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ABSTRACT

The acute oral toxicity of guanidine nitrate was determined in male and female ICR mice by using the oral gavage single-dose method. The median lethal dose was 1105 mg/kg for male mice and 1028 mg/kg for female mice. Clinical signs included behavioral changes, hunched posture, and changes in reflex activity. Behavioral signs observed were irritability, inactivity, disoriented condition, hyperactivity, jumping, tremors, twitching, head tilt, catalepsy, and ataxia. Reflex activity affected by guanidine nitrate administration included depressed grasping and righting reflexes and changes in the startle reflex. The lethality and clinical signs were observed primarily during the first 24 hours after dosing. These results place guanidine nitrate in the slightly toxic category.

KEY WORDS: Acute Oral Toxicity, Guanidine Nitrate, Mouse, Propellants, Nitroguanidine

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PREFACE

TYPE REPORT: Acute Oral Toxicity GLP Study Report

TESTING FACILITY:

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Letterman Army Institute of Research
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SPONSOR:

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GLP STUDY NUMBER: 84002

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PATHOLOGIST: LTC Lance O. Lollini, VC, Diplomate,
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DATA MANAGER: Carolyn M. Lewis, MS

REPORT/DATA MANAGEMENT: A copy of the final report, study
protocol, retired SOPs, raw data,
analytical, stability, and purity
data of the test compound, tissues,
and the test compound will be
retained in the LAIR Archives.

TEST SUBSTANCE: Guanidine Nitrate

INCLUSIVE STUDY DATES: 14 March - 17 April 1984

OBJECTIVE: The objective of this study was to determine the
acute oral toxicity of guanidine nitrate in male
and female Institute of Cancer Research (ICR) mice.

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SIGNATURES OF PRINCIPAL SCIENTISTS AND MANAGERS
INVOLVED IN THE STUDY

We, the undersigned, declare that GLP Study 84002 was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.

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REPLY TO
ATTENTION OF

SGRD-ULZ-QA (73-1n)

24 June 1988

MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance for GLP Study 84002

1. I hereby certify that in relation to LAIR GLP Study 84002, the following inspections were made:

4 January 1984	-	Protocol Review
27 March 1984	-	Necropsy

2. The raw data for this study and the report entitled "Acute Oral Toxicity of Guanidine Nitrate in Mice," Toxicology Series 96, were audited on 22 April 1987 and 17 May 1988.

Carolyn M. Lewis
CAROLYN M. LEWIS, MS
C, Quality Assurance

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Acute Oral Toxicity of Guanidine Nitrate in Mice--
Brown *et al*

INTRODUCTION

Guanidine nitrate is an intermediate product in the synthesis of nitroguanidine. Nitroguanidine is a primary component of US Army triple-base propellants and is now being produced in a Government-owned contractor-operated ammunition plant. The US Army Biomedical Research and Development Laboratory (USABRDL), as part of its mission to evaluate the environmental and health hazards of military-unique propellants generated by US Army munitions-manufacturing facilities, conducted a review of the nitroguanidine data base and identified significant gaps in the toxicity data (1). The Division of Toxicology, LAIR, was tasked by USABRDL to develop a genetic and mammalian toxicity profile for nitroguanidine, related intermediates/by-products of its manufacture, and its environmental degradation products.

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Objective of Study

The objective of this study was to determine the acute oral toxicity of guanidine nitrate in male and female Institute of Cancer Research (ICR) mice.

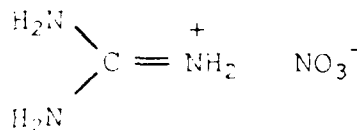
MATERIALS

Test Substance

Chemical name: Guanidine Nitrate

Chemical Abstract Service (CAS) Registry No.: 506-93-4

Molecular structure:



Molecular formula: $\text{CH}_6\text{N}_5\text{O}_3$

Other test substance information is presented in Appendix A.

Vehicle

The suspension vehicle for guanidine nitrate was 0.2% methylcellulose and 0.4% polyoxyethylene-(20)-sorbitan monooleate Tween®80 (MC-TW80) in sterile water for injection. The methylcellulose (lot 12F-0478, expiration date, December 1986) was obtained from Sigma Chemical Company, St. Louis, MO. The Tween®80 (lot 713137, expiration date, December 1986) was obtained from Fisher Scientific Company, Chemical Manufacturing Division, Fair Lawn, NJ. The sterile water for injection (lot 426-27, expiration date, March 1986) was obtained from Cutter Medical Laboratory, Inc., Emeryville, CA.

Animal Data

Seventy-two male and 75 female ICR mice from Harlan Sprague-Dawley, Inc, Indianapolis, IN, were studied. They were identified individually with ear tags numbered 84C00193 - 84C00269 (males) and 84C00270 - 84C00341, 84C00343 - 84C00345 (females). Two males and 2 females were selected at random for quality control necropsy evaluation at receipt. Two animals were removed from the study during quarantine. Eighteen of the animals were studied in an Approximate Lethal Dose (ALD) determination. Six animals were transferred to another protocol. The animal weights on receipt (14 Mar 84) ranged from 23 to 31 g. Additional animal data appear in Appendix B.

Husbandry

Mice were caged individually in stainless steel wire mesh cages in racks equipped with automatic flushing dumptanks. The diet, fed *ad libitum*, consisted of Certified Purina Rodent Chow Diet 5002 (lots JAN19842C and FEB02841D; Ralston Purina Company, Checkerboard Square, St Louis, MO); water was provided by linit valves on a central line. The animal room temperature was constantly monitored and maintained in a range from 20.5°C to 23.3°C with a relative humidity range of 44% to 72% with occasional spikes to 80% due to room cleaning. The photoperiod was 12 hours of light per day.

METHODS

Group Assignment/Acclimation

Study mice were randomized into five dose groups of 10 males and 10 females each and vehicle control groups of 5 males and 5 females each. Allocation was accomplished by

using a computer-based stratified weight-biased method. The Beckman TOXSYS® Animal Allocation Program was used in conjunction with a Beckman TOXSYS® Data Collection Terminal. After the initial dosing, additional mice were assigned to female Groups 3 and 4 to define more completely the dose-response relationship. This left female Group 6 with seven animals. The animals were acclimated for 12 days before the day of dosing. During this period they were observed daily for signs of illness.

Dosage Levels

The results of the ALD determination suggested that the median lethal dose (MLD) was between 800 and 1200 mg/kg for both male and female mice. Based on these data, test doses were selected (Table 1).

TABLE 1
Group Dose Schedule

Group	Dose (mg/kg)
1 (vehicle)	0.2 ml MC-TW80
2	708
3	891
4	1121
5	1410
6	1780

Compound Preparation

Five dosing suspensions (102.5, 129.0, 162.5, 204.5, and 258.0 mg/ml) were prepared by mixing an appropriate quantity of guanidine nitrate in 25 ml of MC-TW80 vehicle. The compound was ground to a fine powder with mortar and pestle and then slowly added to warm (50-60°C) MC-TW80. These mixtures were vortexed, stirred, and sonicated to ensure good suspensions were achieved. Suspensions were evaluated for homogeneity and concentration (Appendix A). Samples for analysis were transferred to screwcapped tubes and stored below 0°C before analysis.

Chemical Analysis of Dosing Solution

Guanidine nitrate was stable in aqueous solution for at least ten days (Appendix A). The vehicle was prepared three days before suspension preparation. Dosing began the day following the preparation of the suspension and was completed within two days for all animals except the seven female mice in Group 6. These seven animals were dosed the following week with a freshly prepared compound suspension. Since the accuracy and homogeneity of guanidine nitrate suspensions in MC-TW80 had been demonstrated previously (see data for GLP Protocol 84001 in Appendix A), only the most concentrated suspension (258 mg/ml) was analyzed for homogeneity for this study. Accuracy and homogeneity data are presented in Appendix A.

Test Procedures

This study was conducted in accordance with EPA guidelines (2) and LAIR SOP OP-STX-36 (3). The volume of dosing solution given each animal was based upon the desired dose level and the compound concentration in suspension. The dose level was increased by varying the concentration of each suspension, and animals received calculated volumes based upon weight. Volumes ranged from 0.21 to 0.27 ml in the male and 0.17 to 0.24 ml in female mice. The vehicle control group was given 0.2 ml of the MC-TW80 vehicle. Dosing was performed by oral gavage without animal sedation or anesthesia. Sterile, disposable syringes fitted with 20-gauge, 1-1/2-inch, ball-tipped, stainless steel Perfektum® oral gavage animal tubes (Popper & Sons, Inc., New Hyde Park, NY) were used for dosing. Homogeneity of test suspensions was ensured by vortexing immediately before withdrawal of the dose and by maintaining the test suspensions on heated (37-40°C) magnetic stir plates. All animals were dosed between 1014 and 1136 hours on 27 and 28 March 1984 except for seven female mice which were dosed between 1046 and 1056 hours on 3 April 1984.

Observations

Observations for mortality and signs of acute toxicity were performed daily according to the following procedure: 1) animals were observed undisturbed in their cages, 2) animals were removed from their cages and given a physical examination, and 3) animals were observed after being returned to their cages. On the day of dosing, the mice were checked intermittently throughout the day. Recorded observations were performed approximately 2-3, 4, and 5 hours after the completion of dosing, and daily for the remainder

of the 2-week test period. A second "walk through" observation was performed daily with only significant observations recorded. Body weights were recorded once weekly during the course of the study.

Necropsy

Animals that died during the observation period were submitted for necropsy. Those which survived the 14-day study period were also submitted for necropsy. The animals were terminated by intraperitoneal barbiturate overdose.

Statistical Analysis

Statistical analyses were performed on the study results. Selected lethal doses were derived by Litchfield-Wilcoxon probit analysis (4). The program, EASYGRAPH (Tektronix, Beaverton, OR), on the Data General Computer, Model MV8000, was used to draw the probit curve based on results from the Litchfield-Wilcoxon analyses.

Duration of Study

Appendix C is a complete listing of historical events.

Changes/Deviations

There were fluctuations in the relative humidity in the animal room associated with steam outages that occurred from 1600 to 2200 hours on 31 March 1984 and from 0800 to 2000 hours on 8 April 1984. These days were selected by the building engineer for routine maintenance. The outages did not affect room temperature; however, the relative humidity did reach 70% and 60%, respectively, on these two days. These deviations were within the relative humidity limits set by the ILAR Guide for the Care and Use of Laboratory Animals.

Animals were observed at 2-3, 4, and 5 hours rather than 1, 2, and 4 hours as originally scheduled due to the length of time it took to dose all the animals.

These changes did not affect the outcome of the study.

Raw Data and Final Report Storage

A copy of the final report, study protocols, raw data, retired SOPs, and an aliquot of the test compound will be retained in the LAIR Archives.

RESULTS**Mortality**

Fifty-one (91.1%) of the deaths occurred within 4 hours after dosing. The remaining five deaths occurred within 24 hours after dosing. Table 2 lists the compound-related deaths by group and the percent mortality. Two misdosed female mice (84C00300, 84C00306) from Group 3 (891 mg/kg) were removed from the study and excluded from statistical analysis. Animal 84C00300 was identified as a misdose at dosing while 84C00306 was identified as a misdose at necropsy. Appendix D is a tabular presentation of cumulative mortality.

TABLE 2

Compound-Related Deaths by Group

Group	Dose (mg/kg)	Compound-Related Death/ Number in Group	Percent Mortality
MALE			
1	Vehicle	0/5	0
2	708	0/10	0
3	891	3/10	30
4	1121	5/10	50
5	1410	9/10	90
6	1780	3/10	30
FEMALE			
1	Vehicle	0/5	0
2	708	2/10	20
3	891	8/13*>	61.5
4	1121	9/15*	60
5	1410	6/10	60
6	1780	5/7>>	71.1

*Numbers in Groups 3 and 4 (females) were increased after the first day of dosing in an attempt to define more completely the dose-response relationships.

>Group 3 (females) contained two additional animals which were misdosed and therefore excluded from statistical analysis and removed from the study.

>>Seven females were assigned to Group 6.

Lethal Dose Calculation

Lethal dose values were calculated by the Litchfield-Wilcoxon method of probit analysis. The equation for the probit regression line was: $Y = -19.10 + 7.92 \log X$ for males and $Y = -3.19 + 2.72 \log X$ for females, where X is the dose and Y the corresponding probit value. Lethal doses calculated from the equation for the probit regression line are presented in Table 3. Figures 1 and 2 graphically present the actual data points and the regression line.

TABLE 3

Calculated Lethal Doses (LD) of Guanidine Nitrate

Effect Level	Dose (mg/kg)	95% Confidence Limits (mg/kg)
MALES		
LD10	762	(603, 963)
LD50	1105*	(957, 1276)
LD90	1603	(1268, 2027)
FEMALES		
LD10	348	(103, 1173)
LD50	1028*	(752, 1404)
LD90	3036	(901, 10,231)

*Median Lethal Dose

Clinical Observations

The most frequently observed categories of clinical signs in animals administered guanidine nitrate were behavioral disturbances (51 of 105 animals dosed), hunched posture (30 of 105), and changes in reflex activity (26 of 105). Behavioral signs included irritability, inactivity, disoriented condition, hyperactivity, jumping, hypertonia, tremors, twitching, head tilt, catalepsy, or ataxia. Changes in reflex activity include depressed grasping and righting reflexes and changes in the startle reflex. Although clinical signs were observed at each dose level, there was no clear dose-response relationship for severity or duration of the symptoms.

FIGURE 1: Dose Mortality Curve for Guanidine Nitrate in Male Mice

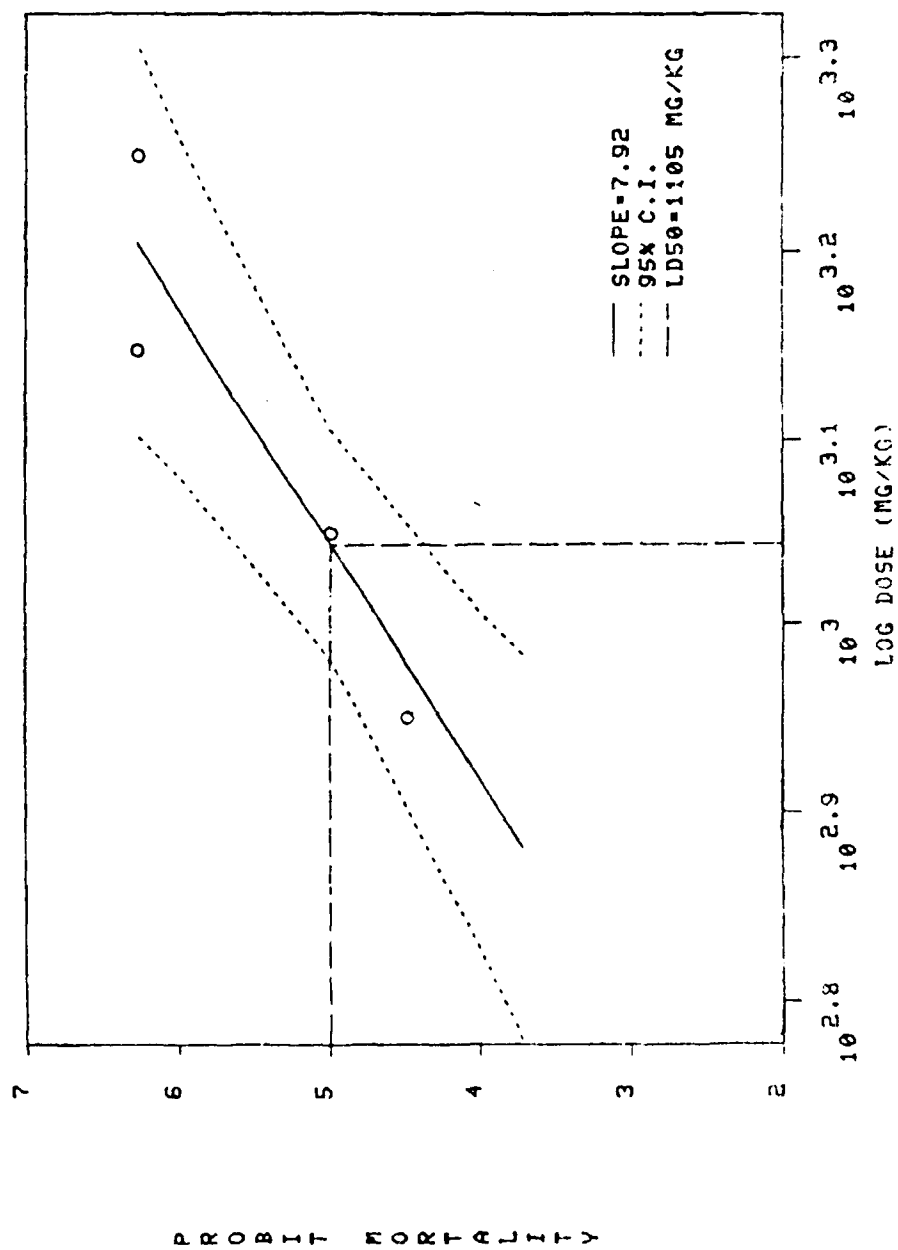
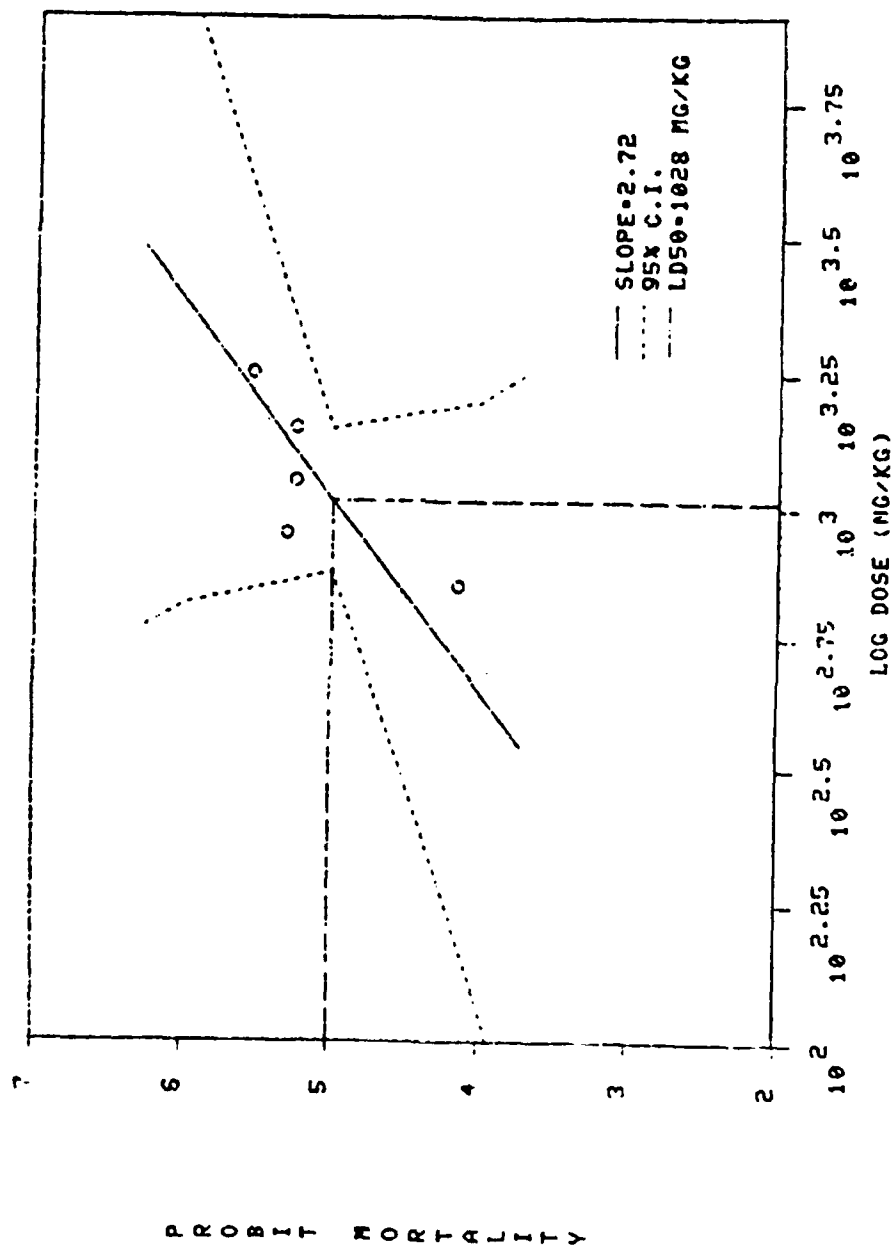


FIGURE 2: Dose Mortality Curve for Guanidine Nitrate in Female Mice



Thirty female mice died during the study. Of the 25 surviving female mice, 17 were normal within 24 hours and another 7 mice were normal within 48 hours. Twenty-six male mice died during the study. Of 24 surviving males, 14 were normal within 24 hours, and 8 other mice were normal within 48 hours. All vehicle control animals survived until study termination at 14 days.

The term "disorientation" describes a behavior pattern in which the animal appeared dazed and confused in response to external stimuli. "Disoriented" animals were observed sitting in their cages with vacant stares and exhibiting little or no response to noise, movement of their cages, or handling. Their movements were hesitant and appeared random except upon perceiving a threat which elicited grossly exaggerated escape movements. Table 4 contains a summary of clinical observations. Appendix E contains individual animal histories.

Weight gains of survivors were not affected by dosing. Table 5 presents the mean body weights by groups. Appendix F contains individual weight tables.

Gross Pathology Observations

Vehicle control animals were normal (NLR = no lesion recorded) by gross examination. The mortalities in the groups which received compound appear to have been caused by the test compound except for one female (84C00306) identified as misdosed. Test compound-related lesions were found only in the respiratory system. The lungs of 19 females and 10 males were congested. A single male in Group 6 (1780 mg/kg) had multiple petechiae in the glandular mucosa of the stomach which was considered an incidental finding. The veterinary pathologist's report appears at Appendix G.

TABLE 4

Incidence Summary for Clinical Observations in Mice
Administered Guanidine Nitrate

Category of Clinical Signs	Group Dose (mg/kg) (N=)	1 Vehicle 5	2 708 10	3 891 10†	4 1121 10†	5 1410 10	6 1780 10†
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MALES

Respiratory*	1	1	0	1	3	2
Gastrointestinal>	1	1	3	1	2	1
Behavioral>>	2	6	6	4	4	3
Skin<	1	1	2	3	1	1
Hunched Posture	0	2	2	0	3	3
Reflex<<	0	2	3	1	3	2
Other#	1	0	1	2	0	0
Normal	1	3	0	1	0	0

FEMALES

Respiratory*	0	0	3	3	2	1
Gastrointestinal>	1	3	3	1	0	1
Behavioral>>	3	8	6	7	5	2
Skin<	1	1	0	2	0	0
Hunched Posture	3	6	2	7	3	2
Reflex<<	1	4	3	4	3	1
Other	0	0	1	0	0	0
Normal	1	0	0	0	0	0

†Number of females in Group 3 was 13, in Group 4 the number was 15, and in Group 6, the number was 7.

*Includes changes in rate, depth, and/or regularity.

>Includes increased salivation, diarrhea, retching movements, or stains on body.

>>Includes irritability, inactivity, disoriented condition, hyperactivity, jumping, hypertonia, tremors, twitching, head tilt, catalepsy, or ataxia.

<Includes alopecia and rough coat.

<<Includes depressed grasping and/or righting reflexes, and changes in the startle reflex.

#Includes prostration, tonic convulsions, and squinting.

TABLE 5
Mean Body Weights In Grams \pm S.E. †

Dose (mg/kg)	At Receipt	Dosing Day	Day 7	Day 14*
MALES				
Vehicle	28.2 ± 0.5 (5)	34.2 ± 0.7 (5)	35.6 ± 0.8 (5)	36.4 ± 0.4 (5)
708	28.1 ± 0.5 (10)	33.5 ± 0.6 (10)	39.4 ± 1.6 (10)	35.3 ± 0.4 (10)
891	28.0 ± 0.4 (10)	34.7 ± 0.6 (10)	37.3 ± 1.3 (7)	36.3 ± 0.9 (7)
1121	26.6 ± 0.5 (10)	34.5 ± 0.8 (10)	40.0 ± 2.2 (5)	36.8 ± 0.7 (5)
1410	28.0 ± 0.6 (10)	34.0 ± 0.6 (10)	35.0 (1)	37.0 (1)
1780	27.6 ± 0.7 (10)	33.7 ± 0.6 (10)	33.0 (1)	35.0 (1)
FEMALES				
Vehicle	25.6 ± 0.3 (5)	28.4 ± 0.9 (5)	30.6 ± 2.6 (5)	28.8 ± 0.9 (5)
708	26.6 ± 0.4 (10)	28.4 ± 0.6 (10)	32.5 ± 2.1 (8)	29.9 ± 0.5 (8)
891	26.5 ± 0.3 (15)	28.1 ± 0.5 (15)	33.8 ± 2.8 (5)	28.2 ± 1.4 (5)
1121	26.1 ± 0.3 (15)	27.7 ± 0.3 (15)	33.2 ± 2.2 (6)	29.0 ± 0.7 (6)
1410	27.2 ± 0.6 (10)	28.5 ± 0.5 (10)	28.0 ± 0.7 (4)	28.8 ± 1.0 (4)
1780	27.3 ± 1.6 (4)	28.6 ± 1.1 (7)	28.0 ± 2.0 (2)	26.5 ± 1.5 (2)

†Number in parenthesis = number of animals.

*Weight after a 4-6 hr fast in preparation for necropsy.

DISCUSSION

The calculated MLD for guanidine nitrate was 1105.0 mg/kg in male ICR mice and 1028.0 mg/kg in female ICR mice. These MLD values are within the slightly toxic range (5).

Guanidine nitrate appeared to have a pharmacologic-toxicologic effect primarily on the central nervous and somatomotor systems as supported by the clinical signs data; however, no correlated gross pathological findings were observed. Guanidine has been reported to increase the amplitude of the neuromuscular end-plate potential by increasing the quantity of acetylcholine released from the nerve endings by a single nerve impulse (6). Because of its ability to augment neuromuscular transmission, the hydrochloride form of guanidine has been used to treat myasthenia gravis (6). More recently, guanidine hydrochloride has been used in the treatment of botulism (7). Since the botulism toxin blocks the release of acetylcholine at the neuromuscular junction, the therapeutic effect of guanidine hydrochloride is attributed to a facilitated release of acetylcholine from remaining efflux sites not blocked by the toxin (8). This proposed mechanism of action is consistent with the listing of guanidine as a striated muscle stimulant (9). It is also consistent with the clinical signs reported in this study.

CONCLUSIONS

The calculated MLD values for guanidine nitrate were 1105.0 mg/kg for male ICR mice and 1028.0 mg/kg for female ICR mice. The lethality and clinical signs were observed primarily during the first 24 hours after dosing. These results indicate that guanidine nitrate is a slightly toxic compound (5).

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Appendix A: CHEMICAL DATA

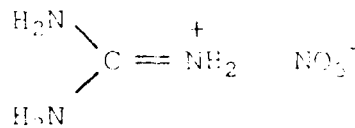
Chemical Name: Guanidine Nitrate

Lot Number: 123820

Chemical Abstracts Registry Number: 506-93-4

LAIR Code: TP030

Chemical Structure:



Molecular Formula: $\text{CH}_6\text{N}_3\cdot\text{NO}_3$

Molecular Weight: 122.1

Physical State: White crystalline powder

Melting Point: 214°C^1

Analytical Data:

Infrared spectrophotometry was performed, and the spectrum obtained² was identical to the Sadtler spectrum³ for Guanidine Nitrate. Major absorption peaks were observed at 3400 (broad), 3200, 1665, 1575, 1400, 1385, and 825 cm^{-1} . The grade of material obtained for this study is referred to as the Ultralog Grade by the manufacturer. The label on the bulk container states that the purity is at least 99.5%.

Source: Chemical Dynamics Corporation
Hadley Road, P.O. Box 395
South Plainfield, NJ

¹Windholz M, ed., The Merck Index, 9th ed., Rahway, NJ: Merck and Co., Inc., 1976: Monograph Number 4411.

²Wheeler CR. Nitrocellulose-Nitroguanidine Project. Laboratory Notebook #84-05-010.2, p. 62. Letterman Army Institute of Research, Presidio of San Francisco, CA.

³Sadtler Research Laboratory, Inc., Sadtler standard spectra, Philadelphia: The Sadtler Research Laboratory, Inc., 1961: Infrared Spectrogram #14498.

Appendix A (cont.): CHEMICAL DATA**Analysis of Dosing Solutions/Suspensions and Determination of Stability**

Dosing solutions/suspensions of guanidine nitrate were vortexed to ensure suspension of particulate material and 1-ml samples were removed from the top, middle, and bottom. Samples were transferred to screwcapped tubes and stored below 0°C prior to analysis.

For analysis, dilution of the sample was necessary. The first dilution was accomplished by heating the 1-ml sample to 50°C in a water bath to dissolve suspended material, and then quickly cooling to room temperature. Before the guanidine nitrate could crystallize out of solution, 0.5 ml was transferred to a 50-ml volumetric flask and diluted to volume with water. This worked satisfactorily for all concentrations except the highest (258 mg/ml). This sample was kept at 50°C while the aliquot was removed.

A second dilution of 1:100 was performed for a total dilution of 1:10,000. Aliquots (2 ml) of the final dilution were assayed using a modification of the Voges-Proskauer assay for guanidine.¹ Quantitation was accomplished by measuring the absorbance of a colored guanidine derivative at a wavelength of 535 nm.

For the first analysis, seven samples were chosen that represented the entire range of concentrations used for dosing.² The results indicated that homogeneous suspensions can be prepared up to 258 mg/ml (Table 1). As a result of this determination, all subsequent analyses were performed with pooled samples (i.e., the top, middle, and bottom samples obtained from dosing solutions/suspensions were heated to dissolve suspended material and pooled).³ These results are presented in Table 2. Of the six suspensions prepared, four were determined to be within 5.2% of the target. The concentration of the two 258-mg/ml suspensions, however, showed a deviation of 10.1% and 10.3% below their target value.

¹Micklus MJ, Stein JM. The colorimetric determination of mono- and disubstituted guanidines. *Anal Biochem* 1973; 53:545-553.

²Wheeler CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.2, p 49-51, 1st. Letterman Army Institute of Research, Presidio of San Francisco, CA.

³*Ibid.* p 52, 55-59.

Appendix A (cont.): CHEMICAL DATA

Analytical Method: Stock Solutions and Calibration Plot

Stock Solution - 50 µg/ml in water:

The stock solution was prepared by weighing out 50.0 mg of guanidine nitrate and transferring this amount to a 1000-ml volumetric flask. The compound was diluted to volume with water and mixed well.

Standard Curve (calibration plot):

To generate the standard curve, two ml of guanidine nitrate solution were prepared at a variety of concentrations as follows:

Final Concentration	ml of stock added to ml of water	
2 µg/ml	0.08 ml stock	1.92 ml water
5	0.20	1.80
10	0.40	1.60
15	0.60	1.40
20	0.80	1.20
25	1.00	1.00
30	1.20	0.80

Appendix A (cont.): CHEMICAL DATA

Calculations:

Linear regression was used to calculate the standard curve. In all cases a correlation coefficient (r) of greater than 0.999 was obtained.

$$\text{assay value} = \frac{\mu\text{g/ml found} \times \text{dilution factor} \times A}{(\text{mg/ml}) \quad 1000}$$

where $\mu\text{g/ml}$ found is determined by linear regression

dilution factor = 10,000

A = 1 for all samples except those obtained from suspensions of guanidine nitrate at 258 mg/ml. For these samples, A has a value of 1.0101 which accounts for the change in volume on going from room temperature to 50°C. This factor was calculated by dividing the volume of 1 kg of water at 50°C by the volume of 1 kg of water at 20°C. Data on water volume as a function of temperature were obtained from the CRC Handbook of Chemistry and Physics, 64th ed., R.C. Weast, ed., 1983, CRC Press, Boca Raton, FL, p. F-5.

Stability:

The stability of guanidine nitrate in aqueous solution is demonstrated by the absorbance values obtained for a standard solution containing 20 $\mu\text{g/ml}$ of guanidine nitrate. This solution was prepared on 25 May and kept at room temperature over the period of analysis. From 25 May to 6 June, four assays of this solution were performed yielding statistically identical absorbance values.¹ Since the Voges-Proskauer assay is specific for unsubstituted and mono-substituted guanidines, the data demonstrate that aqueous solutions of guanidine nitrate are stable for a period of at least 12 days (Table 3).

¹ Wheeler CR. Nitrocellulose-Nitr. Guanidine Projects. Laboratory Notebook #84-05-010.2, p. 55-57, 59. Letterman Army Institute of Research, Presidio of San Francisco, CA.

Appendix A (cont.): CHEMICAL DATA

TABLE 1: Analysis of Dosing Suspensions for Homogeneity

Date Mixed	Date Analyzed	Source of Sample*	Target Conc. mg/ml	Actual Conc. mg/ml	Target
6 Mar	18 May	T	66 >	67.4	102.1
		M		65.5	99.2
		B		65.7	99.5
		Mean		66.2	100.3
14 Mar	18 May	T	83 >	80.5	97.0
		M		75.7 <	91.2
		B		77.2	93.0
		Mean		77.8	93.7
8 Mar	18 May	T	100	96.0	96.0
		M		99.8	99.8
		B		97.6	97.6
		Mean		97.8	97.8
8 Mar	18 May	T	133	122.2	91.9
		M		131.2	98.6
		B		122.8	92.3
		Mean		125.4	94.3
8 Mar	18 May	T	166	162.5	97.9
		M		165.2	99.0
		B		158.7	95.6
		Mean		162.2	97.7
6 Mar	18 May	T	200	184.5	92.3
		M		188.0	94.0
		B		197.0	98.5
		Mean		189.8	94.9
27 Mar†	18 May	T	258	230.0	89.1
		M		245.2	95.0
		B		224.6	87.1
		Mean		233.3	90.1

*The letters T, M, and B refer to the top, middle, and bottom of the dosing solution/suspension.

>These samples were solutions.

<This sample was originally assayed on 18 May and a low value of 67.9 mg/ml was determined. Reanalysis on 29 May 84 gave a value of 75.7 mg/ml. As a check for consistency, the samples prepared on 6 Mar and 27 Mar were also reanalyzed on 29 May. The values obtained for these samples were within 1% of the respective values obtained on 18 May.

†Samples are from ILP study E4002.

Appendix A (cont.): CHEMICAL DATA

TABLE 2: Verification of Guanidine Nitrate Concentration*

Date Mixed	Date Analyzed	Target Conc. mg/ml	Actual Conc.† mg/ml	% Target
26 Mar	25 May	102.5	98.8 ± 0.7	96.4
		129.0	122.1 ± 0.1	94.7
		162.0	151.9 ± 0.8	93.8
		204.0	193.4 ± 1.5	94.8
		258.0	231.9 ± 1.8	89.9
3 Apr	6 Jun	258.0	231.3 ± 1.3	89.7

*Wheeler CR. Nitrocellulose-Nitroguanidine Projects.
Laboratory Notebook #84-05-010.2, p. 57-59. Letterman Army
Institute of Research, Presidio of San Francisco, CA.

†Mean and standard deviation of three analyses.

TABLE 3: Stability Assay of a 20 µg/ml Standard Solution of Guanidine Nitrate

Date of Analysis	Absorbance Values*
25 May 84	1.74 ± 0.02
29 May 84	1.76 ± 0.05
30 May 84	1.76 ± 0.02
6 Jun 84	1.76 ± 0.02

* Values are mean ± S.D. for three replicates.

Appendix B: ANIMAL DATA

Species: *Mus musculus*

Strain: ICR

Source: Harlan Sprague-Dawley, Inc.
P.O. Box 29176
Indianapolis, IN 46229

Sex: Male and female

Date of birth: Male: 27 January 1984
Female: 27 January 1984

Method of randomization: Weight bias, stratified animal
allocation (SOP OP-ISG-24)

Animals in each group: 10 male and female animals, except:
Group 3 females totaled 14,
Group 4 females totaled 15,
Group 6 females totaled 7,
Group 1 (controls) contained 5/sex.

Condition of animals at start of study: Normal

Body weight range at dosing: 25-39 g

Identification procedures: Ear tag (SOP OP-ARG-1), tag
numbers 84C00198 to 84C00345
inclusive.

Pretest conditioning: Quarantine/acclimation 14-26 Mar 84

Justification: The laboratory mouse has proven to be a
sensitive and reliable system for lethal dose
determination.

Appendix C: HISTORICAL LISTING OF STUDY EVENTS

<u>Date</u>	<u>Event</u>
14 Mar 84	Received 72 male and 75 female ICR mice. Mice were checked for physical condition, sexed, weighed, cervical skin tagged, and individually caged.
14-26 Mar 84	Animals were observed daily during quarantine/acclimation period.
15 Mar 84	Four mice (2 male and 2 female) were submitted for necropsy quality control.
16 Mar 84	Animals were weighed and randomized into dose groups. One animal was removed from the study.
20-21 Mar 84	Eighteen ALD animals were fasted 2-4 hours, weighed, dosed, and observed. One animal was removed from the study.
27,28 Mar, 3 Apr 84	One hundred seventeen animals were fasted 2-4 hours, weighed, dosed, and observed three times during the first 6 hours after dosing.
28 Mar- 16 Apr 84	Animals were observed daily for 14 days in a.m. and p.m.
31 Mar, 8 Apr 84	Steam outages occurred in animal suite.
4,11 Apr 84	Animals were weighed.
10,11 Apr 84	All surviving animals except Group 6 females were fasted 2-4 hours, weighed, and submitted to Necropsy at study termination.
17 Apr 84	Six animals transferred to another protocol.
17 Apr 84	Surviving Group 6 females were fasted 2-4 hours, weighed, and submitted to Necropsy at study termination.

Appendix D: Cumulative Mortality Data*

Dose mg/kg	Animals Dosed	<u>Time after Dosing</u>									
		<u>Hours</u>			<u>Days</u>						
		2	4	6	1	2	3	4	5	6	7-14
MALES											
0	5	0	0	0	0	0	0	0	0	0	0
708	10	0	0	0	0	0	0	0	0	0	0
891	10	0	3	3	3	3	3	3	3	3	3
1121	10	0	5	5	5	5	5	5	5	5	5
1410	10	4	7	8	9	9	9	9	9	9	9
1780	10	6	8	9	9	9	9	9	9	9	9
FEMALES											
0	5	0	0	0	0	0	0	0	0	0	0
708	10	0	2	2	2	2	2	2	2	2	2
891	13	0	7	8	8	8	8	8	8	8	8
1121	15	3	8	9	9	9	9	9	9	9	9
1410	10	2	6	6	6	6	6	6	6	6	6
1780	7	5	5	5	5	5	5	5	5	5	5
Totals		20	51	55	56	56	56	56	56	56	56

*Values are deaths/dose group.

Appendix E: INDIVIDUAL ANIMAL HISTORIES

ABBREVIATIONS USED IN APPENDIX E

Inc. = increased
 Dec. = decreased
 Dep. = depressed

MALES: Vehicle Control

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84C00206	Squinting	March 27-28 April 2-7 April 9-10	Slight Slight Slight
84C00208	Alopecia Perianal stain, yellow Head stain, yellow	April 4-9 April 9-10 April 10	Moderate Slight Slight
84C00213	Inc.resp.rate/depth Inactive	March 27 March 29-30	Slight Slight
84C00214	Normal	N/A	N/A
84C00230	Inactive	March 31 April 1-2	Slight Slight

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALES: 708 mg/kg Guanidine Nitrate

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84C00198	Normal	N/A	N/A
84C00199	Perianal stain, yellow Inactive	March 28 March 28-29	Slight Slight
84C00200	Inactive Dep. grasping reflex	March 28, 30 April 2-11 March 28-29	Slight Slight Slight
84C00201	Normal	N/A	N/A
84C00211	Normal	N/A	N/A
84C00227	Inactive Hunched posture	March 28 March 28	Slight Slight
84C00239	Disoriented Hunched posture Catalepsy Inc. resp. rate Dep. grasping reflex	March 28 March 28 March 28 March 28 March 26 April 4 April 5	Marked Moderate Slight Slight Marked Moderate Slight
84C00247	Irritable	March 29	Slight
84C00256	Rough coat	March 29	Slight
84C00269	Irritable Hyperactive	March 28-29 March 28	Slight Marked

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALES: 891 mg/kg Guanidine Nitrate

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84C00212	Death	March 27	2.8 hr
84C00222	Inactive	March 30	Slight
84C00223	Death	March 27	2.5 hr
84C00228	Rough coat	March 27	Slight
	Irritable	March 27	Moderate
84C00242	Prostrate	March 27	N/A
	Twitching	March 27	Marked
	Tremors	March 27	Slight
	Abdominal stain, yellow	March 27	Marked
		March 28	Moderate
	Inactive	April 1-3	Slight
84C00246	Inactive	March 27	Slight
	Dep. grasping reflex	March 27-28	Slight
	Dep. righting reflex	March 27	Slight
	Hypertonia	March 27	Marked
	Hyperactive	March 27-28	Slight
	Irritable	April 9-10	Moderate
84C00253	Dep. grasping reflex	March 27	Slight
	Diarrhea	March 27	Slight
	Perianal stain, yellow	March 27	Slight
84C00258	Inactive	March 27	Slight
	Twitching	March 27	Slight
	Dep. grasping reflex	March 27	Marked
	Disoriented	March 27	Slight
	Hunched posture	March 27	Slight
	Perianal stain, yellow	March 27	Moderate
84C00260	Death	March 27	3 hr
84C00264	Inactive	March 27	Marked
	Hunched posture	March 27	Marked
	Rough coat	March 27	Slight
	Head tilt	March 27	Slight

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALES: 1121 mg/kg Guanidine Nitrate

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84C00204	Death	March 27	2.1 hr
84C00217	Death	March 27	2.1 hr
84C00219	Disoriented	March 27	Moderate
	Inc. resp. depth	March 27	Moderate
	Inc. resp. rate	March 27	Slight
	Inactive	March 27	Moderate
	Tremors	March 27	Moderate
	Dep. grasping reflex	March 27-28	Slight
	Hypertonia	March 27	Marked
	Convulsion, tonic	March 27	Moderate
	Rough coat	March 27-28	Slight
84C00236	Prostrate	March 27	N/A
	Death	March 27	2.7 hr
84C00238	Inactive	March 27	Slight
84C00244	Normal	N/A	N/A
84C00249	Death	March 27	2.2 hr
84C00257	Death	March 27	2.2 hr
84C00261	Rough coat	March 27	Slight
	Twitching	March 27	Moderate
	Disoriented	March 27	Slight
	Hypertonia	March 27	Moderate
	Neck stain, yellow	March 27	Slight
84C00265	Rough coat	March 27	Marked
		March 28	Slight
	Hypertonia	March 27	Slight

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 1410 mg/kg Guanidine Nitrate

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84C00202	Inactive	March 27	Slight
	Hunched posture	March 27	Slight
	Inc. resp. rate	March 27	Marked
	Inc. startle reflex	March 27	Moderate
	Hypertonia	March 27	Slight
	Twitching	March 27	Marked
	Tremors	March 27	Marked
	Death	March 28	24 hr
84C00210	Death	March 27	1.8 hr
84C00215	Inc. Resp. Depth	March 27	Marked
	Dep. righting reflex	March 27	Moderate
	Dep. grasping reflex	March 27	Marked
	Ataxia	March 27	Marked
	Tremors	March 27	Marked
	Hunched posture	March 27	Moderate
	Death	March 27	3.4 hr
84C00216	Death	March 27	2.1 hr
84C00225	Inc. salivation	March 27	Slight
	Perianal stain, brown	March 27	Slight
	Irritable	March 27	Moderate
84C00232	Death	March 27	1.7 hr
84C00240	Death	March 27	1.8 hr
84C00255	Inc. resp. depth	March 27	Marked
	Dec. resp. rate	March 27	Slight
	Hunched posture	March 27	Moderate
	Rough coat	March 27	Marked
	Retching movements	March 27	Marked
	Inactive	March 27	Slight
	Dep. grasping reflex	March 27	Moderate
	Twitching	March 27	Slight
	Hypertonia	March 27	Slight
	Death	March 27	5.2 hr

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 1410 mg/kg Guanidine Nitrate (cont.)

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84C00266	Death	March 27	2.4 hr
84C00268	Death	March 27	1.7 hr

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 1780 mg/kg Guanidine Nitrate

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84C00205	Death	March 28	2.1 hr
84C00207	Inc. resp. rate	March 28	Slight
	Inactive	March 28	Slight
	Disoriented	March 28	Moderate
	Hunched posture	March 28	Slight
	Death	March 28	2.5 hr
84C00209	Death	March 28	1.8 hr
84C00226	Death	March 28	2 hr
84C00233	Death	March 28	1.7 hr
84C00240	Death	March 28	1.6 hr
84C00245	Hunched posture	March 28	Slight
	Inactive	March 28	Moderate
	Disoriented	March 28	Moderate
	Dep. grasping reflex	March 28	Slight
	Inc. resp. rate	March 28	Slight
	Death	March 28	4 hr
84C00251	Hunched posture	March 28-31	Slight
		April 1	Slight
	Inactive	March 28	Moderate
		March 29-30	Slight
	Rough coat	March 28-30	Slight
	Perianal feces, green	March 28	Moderate
	Dep. grasping reflex	March 30	Slight
84C00252	Death	March 28	1.6 hr
84C00259	Death	March 28	1.7 hr

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALES: Vehicle Controls

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84C00276	Hunched posture	March 27	Slight
	Inactive	March 27	Slight
84C00318	Irritable	March 27-28	Slight
		April 2	Slight
	Hunched posture	March 27	Moderate
	Inactive	March 27	Slight
	Hypertonia	March 27	Slight
	Dep. righting reflex	March 27	Slight
84C00328	Disoriented	March 27	Slight
	Hunched posture	March 27	Marked
	Tremors	March 27	Slight
	Alopecia	March 29-31	Slight
		April 1-3	Slight
84C00329	Normal	N/A	N/A
84C00330	Abdomen stain, yellow	March 27	Slight

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALES: 708 mg/kg Guanidine Nitrate

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84C00273	Death	March 27	2.6 hr
84C00284	Hunched posture	March 27	Slight
	Disoriented	March 27	Slight
	Twitching	March 27	Slight
	Back stain, yellow	March 27	Slight
84C00293	Disoriented	March 27-28	Slight
	Hunched posture	March 27	Slight
	Inactive	March 27	Slight
	Dep. grasping reflex	March 27	Moderate
	Irritable	April 4-10	Moderate
84C00296	Death	March 27	2.7 hr
84C00299	Disoriented	March 27	Moderate
	Irritable	March 27	Moderate
	Hunched posture	March 27	Slight
	Inactive	March 27-28, 30	Slight
84C00301	Inactive	March 27	Slight
	Disoriented	March 27	Slight
	Hunched posture	March 27	Slight
84C00305	Twitching	March 27	Moderate
	Inactive	March 30	Slight
84C00324	Hunched posture	March 27	Marked
	Inactive	March 27, 29-31	Slight
	Rough coat	March 27	Slight
	Tremors	March 27	Moderate
	Perianal stain, brown	March 27	Slight
	Dep. grasping reflex	March 28, 30	Slight

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALES: 708 mg/kg Guanidine Nitrate (cont.)

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84C00327	Hunched posture	March 27	Marked
	Disoriented	March 27	Moderate
	Tremors	March 27	Slight
	Perianal stain, brown	March 27	Slight
	Mouth stain, brown	March 27	Slight
	Dep. grasping reflex	March 27-28	Slight
	Hypertonia	March 27	Slight
84C00336	Inactive	March 29-30	Slight
	Disoriented	March 27	Slight
	Dep. grasping reflex	March 29	Slight

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALES: 891 mg/kg Guanidine Nitrate

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84C00275	Death	March 27	2.2 hr
84C00279	Death	March 27	2.2 hr
84C00281	Death	March 28	2.4 hr
84C00283	Irritable	March 27	Slight
	Disoriented	March 27	Slight
	Inactive	March 30	Slight
84C00287	Hunched posture	March 27	Marked
	Disoriented	March 27	Slight
	Inactive	March 27	Slight
	Twitching	March 27	Slight
84C00291	Inactive	March 28	Moderate
		April 3	Slight
	Twitching	March 28	Slight
	Dep. grasping reflex	March 28	Moderate
	Inc. resp. rate	March 28	Slight
84C00295	Death	March 27	2.2 hr
84C00297	Inactive	March 27	Moderate
	Inc. resp. depth	March 27	Slight
	Twitching	March 27	Marked
	Tremors	March 27	Moderate
	Disoriented	March 27	Marked
	Perianal stain, yellow	March 27	Slight
	Mouth stain, yellow	March 27	Moderate
	Dep. grasping reflex	March 27	Moderate
	Dep. righting reflex	March 27	Moderate
84C00300	Misdose		

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALES: 891 mg/kg Guanidine Nitrate (cont.)

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84C00303	Inactive	March 28	Moderate
	Perianal feces	March 28	Slight
	Hunched posture	March 28	Slight
	Irritable	March 28	Moderate
		March 30	Slight
	Disoriented	March 28	Slight
84C00304	Inactive	March 27	Moderate
	Inc. resp. depth	March 27	Moderate
	Disoriented	March 27	Moderate
	Dep. grasping reflex	March 27	Marked
	Dep. righting reflex	March 27	Slight
	Tremors	March 27	Marked
	Twitching	March 27	Marked
	Retching movement	March 27	Slight
	Prostrate	March 27	N/A
	Death	March 27	5.2 hr
84C00306	Misdose		
84C00307	Death	March 28	2.2 hr
84C00313	Death	March 27	3.3 hr
84C00325	Death	March 27	2.1 hr

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALES: 1121 mg/kg Guanidine Nitrate

Animal	Clinical Signs	Dates (1984)	Severity
84C00270	Hunched posture	March 27	Marked
	Disoriented	March 27	Marked
	Inc. resp. depth	March 27	Moderate
	Jumping	March 27	Moderate
	Death	March 27	4.2 hr
84C00278	Hunched posture	March 27	Marked
	Inactive	March 27	Moderate
	Rough coat	March 27	Slight
	Disoriented	March 27	Moderate
	Diarrhea	March 27	Slight
	Perianal stain, yellow	March 27	Slight
	Dep. grasping reflex	March 27	Moderate
	Hyperactive	March 27	Slight
84C00285	Irritable	April 3	Slight
	Disoriented	March 27	Moderate
	Hunched posture	March 27	Moderate
	Inactive	March 27	Moderate
	Dep. grasping reflex	March 27	Marked
84C00289	Irritable	March 29-31	Slight
	Disoriented	March 27-28	Slight
	Hypertonia	March 27	Moderate
	Irritable	March 27-28	Moderate
		April 3-9	Slight
	Inactive	March 27-28	Slight
	Hunched posture	March 27-28	Slight
84C00292	Death	March 27	1.9 hr
84C00309	Death	March 27	1.8 hr
84C00310	Death	March 28	2.2 hr
84C00311	Death	March 28	2.2 hr
84C00315	Death	March 27	1.8 hr

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALES: 1121 mg/kg Guanidine Nitrate (cont.)

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84C00319	Disoriented	March 27	Marked
	Dep. grasping reflex	March 27	Moderate
	Tremors	March 27	Moderate
	Inc. resp. rate	March 27	Slight
	Hypertonnia	March 27	Slight
	Hunched posture	March 27	Slight
84C00323	Death	March 28	2.1 hr
84C00326	Death	March 27	3 hr
84C00332	Inactive	March 28	Marked
	Hunched posture	March 28	Moderate
	Disoriented	March 28	Moderate
	Gasping	March 28	Slight
	Tachypnea	March 28	Moderate
84C00335	Hunched posture	March 27	Marked
	Rough coat	March 27	Moderate
	Tremors	March 27	Marked
	Disoriented	March 27	Moderate
	Inc. startle reflex	March 27	Slight
	Irritable	March 27-28	Marked
		March 31	Slight
		April 2-7	Moderate
	Hyperactive	March 27-28	Marked
	Inactive	March 31	Slight
		April 5-6	Slight
84C00338	Death	March 28	2.1 hr

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALES: 1410 mg/kg Guanidine Nitrate

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84C00272	Death	March 28	2.1 hr
84C00277	Death	March 28	2.2 hr
84C00286	Death	March 28	1.9 hr
84C00288	Inactive	March 30	Slight
84C00294	Hunched posture	March 28	Slight
	Irritable	March 28	Slight
	Inactive	March 28	Moderate
	Dep. grasping reflex	March 28	Moderate
84C00302	Death	March 28	2 hr
84C00308	Hunched posture	March 28	Moderate
	Inactive	March 28	Moderate
	Inc. resp. rate	March 28	Moderate
	Irritable	March 28	Slight
84C00314	Jumping	March 28	Marked
	Twitching	March 28	Marked
	Inactive	March 28	Marked
	Inc. resp. rate	March 28	Moderate
	Dep. grasping reflex	March 28	Marked
	Death	March 28	3.4 hr
84C00337	Irritable	March 28	Slight
	Disoriented	March 28	Moderate
	Hunched posture	March 28	Slight
	Dep. grasping reflex	March 28	Slight
	Inactive	March 29	Slight
84C00339	Death	March 28	2.1 hr

Appendix E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALES: 1780 mg/kg Guanidine Nitrate

Animal Number	Clinical Signs	Dates Observed (1984)	Severity
84C00271	Death	April 3	1.7 hr
84C00274	Death	April 3	0.7 hr
84C00321	Death	April 3	0.8 hr
84C00340	Diarrhea, green	April 3	Slight
	Inc. resp. rate	April 3	Slight
	Disoriented	April 3	Slight
	Hunched posture	April 3	Moderate
	Twitching	April 3	Slight
	Hypertonia	April 3	Slight
	Irritable	April 11	Slight
84C00343	Dep. grasping reflex	April 3	Moderate
		April 4	Slight
	Hunched posture	April 3	Slight
	Disoriented	April 3	Moderate
	Hypertonia	April 3	Moderate
	Irritable	April 3-4	Slight
84C00344	Death	April 3	0.7 hr
84C00345	Death	April 3	0.6 hr

Appendix F: INDIVIDUAL BODY WEIGHTS**MALES: Vehicle Controls**

Animal Number	Receipt	Dosing	Day 7	Termination Day 14	Weight* Change
84C00206	28†	34	35	36	+2
84C00208	27	35	37	37	+2
84C00213	30	34	37	37	+3
84C00214	28	36	36	37	+1
84C00230	28	32	33	35	+3
.....					
Mean	28.2	34.2	35.6	36.4	
Standard Error	0.5	0.7	0.7	0.4	

*Dosing to termination (after a 3-hr fast).

†Weight is given in grams.

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS

MALES: 708 mg/kg

Animal Number	Receipt	Dosing	Day 7	Termination Day 14	Weight* Change
84C00198	30†	33	45	37	+4
84C00199	29	34	45	36	+2
84C00200	30	33	44	34	+1
84C00201	25	34	44	36	+2
84C00211	28	32	42	34	+2
84C00227	26	30	32	33	+3
84C00239	28	33	34	35	+2
84C00247	29	38	38	37	-1
84C00256	29	33	34	35	+2
84C00260	27	35	36	36	+1
.....					
Mean	28.1	33.5	39.4	35.3	
Standard Error	0.5	0.7	1.6	0.4	

*Dosing to termination.

†Weight is given in grams.

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS

MALES: 891 mg/kg

Animal Number	Receipt	Dosing	Day 7	Termination Day 14	Weight* Change
84C00212	27†	35	Dead		
84C00222	28	34	35	36	+2
84C00223	28	33	Dead		
84C00228	30	36	37	37	+1
84C00242	29	38	39	40	+2
84C00246	27	34	35	36	+2
84C00253	26	35	35	33	-2
84C00258	30	37	NR>	38	+1
84C00260	27	32	Dead		
84C00264	28	33	43	34	+1
.....					
Mean	28.0	34.7	37.3	36.3	
Standard Error	0.4	0.6	1.3	0.9	

*Dosing to determination.
†Weight is given in grams.
>NR = Not Recorded.

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS

MALES: 1121 mg/kg

Animal Number	Receipt	Dosing	Day 7	Termination Day 14	Weight Change
84C00204	26†	32	Dead		
84C00217	27	32	Dead		
84C00219	28	39	39	39	+0
84C00236	29	36	Dead		
84C00238	23	35	36	37	+2
84C00244	26	34	35	35	+1
84C00249	27	38	Dead		
84C00257	28	31	Dead		
84C00261	26	34	44	36	+2
84C00265	26	34	46	37	+2
.....					
Mean	26.6	34.5	40.0	36.8	
Standard Error	0.5	0.8	2.2	0.7	

*Dosing to termination.

†Weight is given in grams.

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS

MALES: 1410 mg/kg

Animal Number	Receipt	Dosing	Day 7	Termination Day 14	Weight* Change
84C00202	26†	34	Dead		
84C00210	31	35	Dead		
84C00215	30	30	Dead		
84C00216	29	36	Dead		
84C00225	29	34	35	37	+3
84C00232	27	33	Dead		
84C00240	26	35	Dead		
84C00255	26	33	Dead		
84C00266	27	37	Dead		
84C00268	29	33	Dead		
.....					
Mean	28.0	34.0	35.0	37.0	
Standard Error	0.6	0.6			

*Dosing to termination.
†Weight is given in grams.

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS

NALEP: 1750 mg/kg

Animal Number	Receipt	Dosing	Day 7	Termination Day 14	Weight Change
84C00205	24*	35	Dead		
84C00207	27	34	Dead		
84C00209	26	35	Dead		
84C00226	30	35	Dead		
84C00233	29	32	Dead		
84C00243	28	32	Dead		
84C00245	26	35	Dead		
84C00251	26	32	33	35	+3
84C00252	30	33	Dead		
84C00259	30	33	Dead		
.....					
Mean	27.6	32.7	33.0	35.0	
Standard Error	0.7	0.6			

*Dosing to termination.

†Weight is given in grams.

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS

FEMALES: Vehicle Controls

Animal Number	Receipt	Dosing	Day 7	Termination Day 14	Weight* Change
FEMALE					
84C00276	26†	31	41	31	0
84C00318	25	29	28	30	+1
84C00328	26	27	27	26	-1
84C00329	26	26	28	28	+2
84C00330	25	29	29	29	0
.....					
Mean	25.6	28.4	30.6	28.8	
Standard Error	0.2	0.9	2.6	0.9	

*Dosing to termination.

†Weight is given in grams.

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS

FEMALES: 708 mg/kg

Animal Number	Receipt	Dosing	Day 7	Termination Day 14	Weight * Change
84C00273	27†	32	Dead		
84C00284	27	28	39	28	+0
84C00293	27	29	44	32	+2
84C00296	28	25	Dead		
84C00299	26	28	28	29	+1
84C00301	28	30	30	31	+1
84C00305	27	27	27	30	+3
84C00324	25	28	30	29	+1
84C00327	24	27	31	31	+4
84C00336	27	30	31	29	-1
.....					
Mean	26.6	28.4	32.5	29.9	
Standard Error	0.4	0.6	2.1	0.5	

*Dosing to termination.

†Weight is given in grams.

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS

FEMALES: 891 mg/kg

Animal Number	Receipt	Dosing	Day 7	Termination Day 14	Weight* Change
84C00275	27†	28	Dead		
84C00279	27	27	Dead		
84C00281	26	25	Dead		
84C00283	26	29	39	29	+0
84C00287	28	30	42	26	-4
84C00291	27	29	30	30	+1
84C00295	27	26	Dead		
84C00297	28	28	30	32	+4
84C00300	25	27	Misdose		
84C00303	25	27	28	24	-3
84C00304	26	32	Dead		
84C00306	25	27	Misdose		
84C00307	28	29	Dead		
84C00313	27	30	Dead		
84C00325	25	27	Dead		
.....					
Mean	26.5	28.1	33.8	28.2	
Standard Error	0.3	0.5	2.8	1.4	

*Dosing to termination.

†Weight is given in grams.

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS

FEMALES: 1121 mg/kg

Animal Number	Receipt	Dosing	Day 7	Termination Day 14	Weight* Change
84C00270	26†	28	Dead		
84C00278	26	29	39	30	+1
84C00285	26	27	37	29	+2
84C00289	26	27	37	28	+1
84C00292	26	27	Dead		
84C00309	27	28	Dead		
84C00310	26	27	Dead		
84C00311	26	28	Dead		
84C00315	25	26	Dead		
84C00319	26	27	28	28	+1
84C00323	27	29	Dead		
84C00326	26	28	Dead		
84C00332	29	31	32	32	+1
84C00335	25	26	26	27	+1
84C00338	24	28	Dead		
.....					
Mean	26.1	27.7	33.2	29.0	
Standard Error	0.3	0.3	2.2	0.7	

*Dosing to termination.

†Weight is given in grams.

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS

FEMALES: 1410 mg/kg

Animal Number	Receipt	Dosing	Day 7	Termination Day 14	Weight* Change
84C00272	30†	30	Dead		
84C00277	27	28	Dead		
84C00286	28	29	Dead		
84C00288	26	29	29	30	-1
84C00294	25	26	26	26	+0
84C00307	29	30	Dead		
84C00308	26	29	29	30	+1
84C00314	27	26	Dead		
84C00337	25	27	28	29	+2
84C00339	29	31	Dead		
.....					
Mean	27.2	28.5	28.0	28.8	
Standard Error	0.6	0.5	0.7	0.9	

*Dosing to termination.
†Weight is given in grams.

Appendix F (cont.): INDIVIDUAL BODY WEIGHTS

FEMALES: 1780 mg/kg

Animal Number	Receipt	Dosing	Day 7	Termination Day 14	Weight Change
84C00271	30†	29	Dead		
84C00274	30	34	Dead		
84C00321	24	29	Dead		
84C00340	25	30	30	28	-2
84C00343	NR>	25	26	25	0
84C00344	NR>	27	Dead		
84C00345	NR>	26	Dead		
.....					
Mean	27.2	28.6	28.0	26.5	
Standard Error	1.6	1.1	2.0	1.5	

†Dosing to termination.

†Weight is given in grams.

>Transferred from a Non-GLP Study, initial quarantine weight not recorded (NR).

Appendix G: PATHOLOGY REPORT

ACUTE ORAL TOXICITY TEST IN MICE OF GUANIDINE NITRATE

GLP Study 84002

History: This study was designed to determine the acute oral toxicity of guanidine nitrate in male and female mice. ICR mice, average weight 30 grams, were divided into six (6) groups with varying numbers per group (see table). After acclimation and randomization, these animals were dosed by oral gavage as follows:

- Group 1 - 0.2 cc vehicle (methylcellulose/Tween® 80)
- Group 2 - 708.0 mg/kg guanidine nitrate
- Group 3 - 891.0 mg/kg guanidine nitrate
- Group 4 - 1121.0 mg/kg guanidine nitrate
- Group 5 - 1410.0 mg/kg guanidine nitrate
- Group 6 - 1780.0 mg/kg guanidine nitrate

Twenty-six (26) males and thirty-one (31) females died within the first 24 hours after dosing. The remaining mice survived until completion of the 14-day post-treatment period. These animals were killed by intraperitoneal exposure to pentobarbital. All animals were necropsied.

Gross Necropsy Findings:

Males: Twenty-six of the 50 males exposed to guanidine nitrate died within the first 24 hours after dosing. The lungs of ten of these males were congested. A single male in Group 6 (1780.0 mg/kg) also had multiple petechiae in the glandular mucosa of the stomach. The remaining mice had no gross lesions at necropsy.

Females: Thirty-one of the 56 females exposed to guanidine nitrate died within the first 24 hours after dosing. The lungs of nineteen of these females were congested. The remaining mice had no gross lesions at necropsy. One animal in Group 3 had a clear, red, nonclotting thoracic fluid (less than 1 cc).

No tissue was saved for microscopic evaluation.

Appendix G (cont.): PATHOLOGY REPORT

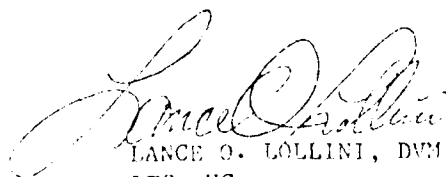
TABLE 1 - Males

Group Number	1	2	3	4	5	6
Animals/Group	5	10	10	10	10	10
Deaths	0	0	3	5	9	9
% Deaths	0	0	30	50	90	90
Survivors	5	10	7	5	1	1
Lesions	0	0	1	3	2	4
Lung -congestion			1	3	2	4
Stomach - mucosal petechiae						1

TABLE 2 - Females

Group Number	1	2	3	4	5	5
Animals/Group	5	10	14	15	10	7
Deaths	0	2	9	9	6	5
% Deaths	0	20	64	60	60	71
Survivors	5	8	5	6	4	2
Lesions	0	1	7	6	5	0
Lung - congestion		1	7	6	5	
Thoracic fluid			1			

Summary: The diffusely reddened lungs were believed to be due to vascular congestion -- these animals had been convulsing prior to death. The female that had thoracic fluid may have represented iatrogenic deposition of compound into the thorax via esophageal puncture. The gastric mucosal lesions in one male were considered to have been an incidental, unrelated finding. A dose effect was clearly present and the difference noted between the males and females possibly was due to sexual dimorphism.



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Chief Pathology Services Group

13 August 1984

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